REMARKS

A. Introductory remarks

Claims 1, 5, 7, 12-15, 17, 18, 21 and 130-138 were pending before the Office. Applicants have amended claims 1, 5, 7, 12-15, 17, 18, 21 and 131-138 and canceled claims 130-131, without prejudice. Claim 139 is added. No claims are withdrawn. Accordingly, claims 1, 5, 7, 12-15, 17, 18, 21 and 132-139 will be pending upon entry of these amendments.

The amendments have been made solely to claim more fully the invention and/or to expedite prosecution of the present application and should in no way be construed as an acquiescence to any of the Examiner's rejections in the Office action issued in the present application. Applicants reserve the right to pursue the subject matter of the claims as originally filed or similar claims in one or more subsequent applications.

Support for the amendments and new claims can be found throughout the application, including the specification, drawings, examples and claims, as originally filed. No new matter has been added by these amendments.

Support for the claim amendments:

Exemplary support for the claim amendments can be found as follows. Applicants emphasize that the support summarized below is by example only and that additional support can be found throughout the specification, drawings and original claims as-filed. No new matter has been added by the claim amendments presented in this Response. Supported is cited in reference to specific paragraphs of the published application, U.S. Published Application No. US 2006/0166189.

With respect claim 1, support "rapid vaccinia neutralization assay" and "wherein the assay can be completed within 24 hours," can be found, for example, a page 1, beginning line 26, which recites an "antibody binding to a pathogen that disallows productive infection by the pathogen is called neutralization." Nuetralization, as taught in the "background of the invention" section is a well known term in the field of immunology, and consists of a decrease in the infectious titre of a viral preparation following its exposure to antibodies or other nuetralizing agents. The assay of the invention is rapid as it can be completed within 24 hours, which is supported, for example, in paragraph 30 ("It is demonstrated herein that the assay is rapid (24 hr), of equal sensitivity to PRNT assays, reproducible, objective, and easy to transfer.") and paragraph 31 ("For example, it has been

demonstrated that the β-Gal reporter gene assay, using a recombinant vaccinia virus vSC56 is rapid (24 hr), sensitive, reproducible, and produces very similar results to those obtained in two different PRNT assays."). Applicants wished to clarify that the method of the invention is a neutralization assay.

Also in claim 1, support for "incubating *in vitro* a mixture comprising at least one cell, a vaccinia virus comprising a reporter gene encoding an enzyme, and a candidate antibody under conditions wherein the vaccinia virus can invade the cell" is supported, for example, in the Description at paragraphs 9, 16, 30, 62-71 and in the Examples, for instance, at paragraphs 110-119, and paragraph 135.

Support for "detecting the activity of the enzyme within the cell" can be found, for example, in the Description, for instance, at paragraph 70, and in the Examples, for instance, in Example I at paragraph 117.

Support for "wherein a decrease in the enzyme activity in the cell due to the candidate antibody, relative to a control cell having not been contacted with the candidate antibody, indicates that the candidate antibody decreases invasion of the cell by the vaccinia virus," can be found, for example, in the Description, for instance, at paragraph 64.

Support for "wherein the *in vitro* detection of decreased invasion correlates positively with the *in vivo* protective efficiency of the candidate antibody against lethal infection with a vaccinia virus vaccine," can be found in the Description, for example, at paragraph 33, which expressly teaches that "The difference in neutralization titer observed in the β-Gal assay correlated positively with significant difference in the protective efficiency against lethal infection of SCID mice with vaccinia (Wyeth)."

As to claims 5 and 7, the vSC56 and vSC8 vectors are described, for example, in the Description, for instance, at paragraphs 31 and in the Examples, for instance, at paragraph 111.

As to claims 12 and 13, support for the reporter genes can be found, for example, in the Description at paragraph 16.

As to claim 15, support can be found, for example, in the Description at paragraph 69.

As to claim 136, support can be found, for example, in Example at paragraph 134.

Support for the amendment to claim 131 and as to new claim 139 can be found, for example, at paragraph 33.

In addition, each of the pending claims has been amended to the refer to the "assay" of a prior claim instead of the "method."

In view of at least the above, Applicants believe that the claim amendments are fully supported by the present specification and do not constitute new matter.

B. The claim rejections under 35 U.S.C. 112 are overcome

Claims 130, 132 and 136 stand rejected under 35 U.S.C. 112, second paragraph, as allegedly failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. Specifically, claims 130 and 132 were rejected on the basis of the recitation of "the assay" allegedly in the absence of antecedent basis. Claim 136 was rejected for the term "classic" as allegedly being vague. Without wishing to acquiesce the grounds of the rejection, applicants have cancelled claim 130. With regard to claim 132, Applicants note that the preamble of claim 1 was clarify to indicate that the recited method is a nuetralization assay. Thus, the rejection of claim 132 is overcome. Claim 136 was amended to delete the term "classic" as the term is unnecessary to particularly point out and claim the subject matter of claim 136. Applicants respectfully request reconsideration and withdrawal of the present 112 rejection.

C. *The claim rejections under 35 U.S.C. § 103(a) are overcome*

Based on Applicant's arguments, the Examiner has withdrawn the previous Section 103 rejection of claims over the combination of Dominguez et al. (Journal of Immunological Methods, 1998, 220:115-221, "Dominguez") and Hooper et al. (U.S. Patent No. 6,451,309, "Hooper"), as well as the Section 103 rejection of claims over Dominguez and Hooper in view of Engelmayer et al. (The Journal of Immunology, 1999, 163:6762-6768, "Engelmayer").

In this Office Action, the Examiner newly rejects claims 1, 5, 7, 12-15, 17, 18, 21, 130, 131 and 136 under 35 U.S.C. 103(a) as allegedly being unpatentable over Hooper in view of Auewarakul et al. (Asian Pacific Journal of Allergy and Immunology, 2001, 19:139-144, cited in IDS filed 3/29/07, "Auewarakul") and Dominguez. In addition, claims 132 and 135 have been rejected under 35 U.S.C. 103(a) as allegedly being unpatentable Hooper in view of Auewarakul and Dominguez, as applied to claim 1, and in further view of Briskin et al. (U.S. Patent No. 6,319,675)

and BD Biosciences (Introduction to Flow Cytometry: A Learning Guide, Manual Part Number: 11-11032-01, April, 2000, "BD Biosciences"). Further, claims 133 and 134 have been rejected under 35 U.S.C. 103(a) as allegedly being unpatentable Hooper in view of Auewarakul and Dominguez, as applied to claim 1, and in further view of BD Biosciences.

The Examiner argues that it would have been obvious to modify Hooper's method of producing and identifying therapeutic anti-vaccinia monoclonal antibodies in mice using a reporter virus in a neutralization assay using the flow cytometric method described by Auewarakul for testing anti-HIV antibodies using a modified HIV-GFP reporter system. Moreover, the Examiner contends that one of ordinary skill in the art would have been capable of and motivated to substitute Auewarakul's HIV-GFP reporter system with the recombinant GFP-expressing vaccinia system described in Dominguez as a suitable reporter virus to practice the claimed invention.

Applicants respectfully disagree and traverse the rejection.

Applicants respectfully submit that none of the cited references, taken alone or in any combination, teach or fairly suggest all of the elements of the instantly claimed invention. Further, even if the references suggested all of the claimed elements, which is not admitted here, there would not have been any reason to combine the references, nor would there have been any reasonable expectation that the combination would have produced the invention. Accordingly, the present invention would not have been obvious in view of the correct standard for obviousness.

The correct obviousness standard is still provided by *Graham v. John Deere Co.,* 338 U.S. 1, 148 USPQ 459 (1966), which was reaffirmed by *KSR International Co. v. Teleflex Inc.,* 127 S.Ct. 1727, 82 USPQ2d 1385 (2007) as providing the correct analytical framework for determining obviousness. Under *Graham*, obviousness is a question of law based on underlying factual inquires that address (1) the scope and content of the prior art, (2) the differences between the claimed invention and the prior art, and (3) the level of ordinary skill in the pertinent art. Additionally, the Supreme Court in *KSR* required a "clear articulation of the reason(s) why the claimed invention would have been obvious" and that such reason "supporting a rejection under 35 U.S.C. 103 should be made explicit."

Here, the Office Action indicates that the combination of references is proper because there exists some teaching, suggestion, or motivation in the prior art that would have led the skilled

artisan to modify or combine the cited prior art to arrive at claimed invention. For the obviousness rejection to be proper, the prior art references or their combination must be shown to ***teach or suggest all the claim limitations***. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974). In addition, for each rejection, the Office must articulate (1) a ***teaching, suggestion or motivation to combine*** the references and (2) ***a finding of a reasonable expectation of success***. Accordingly, prior art which teaches or suggests all the claim limitations and is enabling as to same may be modified or combined to reject claims as *prima facie* obvious so long as there is a reasonable expectation of success and a reason to combine. Such is not the case here for the reasons that follow.

The present invention

The presently claimed invention is directed to a novel *in vitro* neutralization assay based on the use of a recombinant vaccinia virus vector that is engineered to express an enzyme-based reporter gene, such as β-galactosidase and which is rapid in that it can be conducted within 24 hours. Specifically, claim 1 is directed to a method comprising incubating a mixture comprising at least one cell, a vaccinia virus comprising a reporter gene encoding an enzyme, and a candidate antibody under conditions wherein the vaccinia virus can invade the cell. The method then proceeds by detecting the activity of the enzyme within the cell. The claim further recites that a decrease in the enzyme activity in the cell due to the candidate antibody, relative to a control cell having not been contacted with the candidate antibody, indicates that the candidate antibody decreases invasion of the cell by the vaccinia virus. The encoded reporter enzyme can be β-galactosidase, luciferase, peroxidase, alkaline phosphatase, or xanthine oxidase, and preferable can be β-galactosidase or luciferase. As reflected in claim 131, the inventive method is predictive of viral lethality in a mouse model *in vivo*. Further, as reflected in claim 139, the mouse model is a SCID mouse model.

As taught in the specification, the claimed method is the only validated alternative method to the well-known, but labor intensive, plaque reduction neutralization assay (PRNT). As taught in the specification, results obtained using the instantly claimed method, are comparable to results obtained with the classic PRNT vaccinia neutralization assays (e.g., paragraphs 30, 134). Moreover, the specification, the high throughput technology makes the claimed method highly sensitive (e.g.,

paragraphs 30, 135), easier to conduct (even with small volumes), faster, easy to transfer to other laboratories (e.g., paragraphs 30, 121-122), and good reproducibility (e.g., paragraphs 30, 136).

Applicants note that the significance and importance of the present method can readily be understood not only on the basis of the specification itself, but also on the use and/or adaptation of the inventive method in numerous scientific publications coming after the time of the invention and which specifically refer to and/or utilize the assay of the invention. For example, the presently claimed method was first published by the inventors in Manischewitz et al., "Development of a Novel Vaccinia-Neutralization Assay Based on Reporter-Gene Expression," The Journal of Infectious Diseases, August 1, 2003, 188, pages 440-448, published well after the filing of Applicants' priority application (herein as "Manischewitz"). The data in Manischewitz corresponds to the Examples of the present application. Manischewitz emphasizes the significance of the present invention:

> We have established and standardized a novel assay to measure vaccinia neutralization. It is based on the expression of a reporter gene encoding the bacterial β-galactosidase (β-Gal) enzyme, under the control of a synthetic early/late promoter. When selecting a recombinant vaccinia expressing a reporter gene for a single round of infection, we reasoned that an enzyme-encoding gene would provide augmented sensitivity, compared with other reporter genes, since each enzyme molecule converts multiple substrate molecules within the time frame of the assay. We demonstrate that the new assay is rapid (24 h), is of equal sensitivity to PRNTs, is reproducible, and is objective. The new assay is high-throughput assay and is amenable to validation. These characteristics should make the assay easy to transfer to clinical laboratories, for the evaluation of multiple samples from clinical trials. (pages 440-441)

Manischewitz further teaches the advantages:

> Therefore, in vitro vaccinia-neutralization assays provide a good estimate of the vaccinia-neutralization potential of immune serum samples, and our assay provides a rapid, easy-to-validate, high-throughput alternative to the PRNTs. (page 447)

Manischewitz still further teaches:

> These recombinant viruses provide us with excellent tools to aid in the assessment of the protective activities, both in vitro and in vivo, of new anti-vaccinia reagents. (page 448)

Further, in a later publication co-authored by the inventor, Kennedy et al., "Statistical approach to estimate vaccinia-specific neutralizing antibody titers using a high-throughput assay," Clin Vaccine Immunol. 2009 Aug;16(8):1105-12 (hereinafter as "Kennedy"), the references makes clear that the assay of the inventioned has been widely used since its first appearance in Manischewitz. Kennedy teaches that:

> The β-Gal-based based assay was initially described in 2003 and is as sensitive as PRNTS but is more rapid and easily adapted to high throughput and has an objective readout conducive to large-scale statistical analyses (35). Since its initial description, it has been used for a number of studies, including a comparison of Dryvax (Wyeth Laboratories, Inc.) and modified vaccinia virus Ankara immunization in a mouse study (37), the detection of vaccinia virus-specific Ab titers in samples from human immunodeficiency virus-positive adults (29), the evaluation of different vaccination sittes for human subjects (53), a nonhuman-primate study showing that Ab responses are sufficient for protection against monkeypox (13,14), and the evaluation of a subunit recombinant vaccine for use in protection against monkypox (22).

The majority of these references cited by Kennedy involved the inventors. However, a search of the public literature database of references citing to Manischewitz can be perfomed to identify those references that also involve the β-Gal neutralization assay of the invention. For example, the following ten (10) publications specifically cite to Manischewitz and/or its β-Gal neutralization assay (only 2 (Nos. 2, 3) of which are co-authored by the inventor):

> 1: von Krempelhuber et al., "A randomized, double-blind, dose-finding Phase II study to evaluate immunogenicity and safety of the third generation smallpox vaccine candidate." Vaccine. 2010 Feb 3;28(5):1209-16. Epub 2009 Nov 25;
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> 2: Meseda et al., "Comparative evaluation of the immune responses and protection engendered by LC16m8 and Dryvax smallpox vaccines in a mouse model," Clin Vaccine Immunol. 2009 Sep;16(9):1261-71. Epub 2009 Jul 15;
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> 3: Kennedy et al., "Statistical approach to estimate vaccinia-specific neutralizing antibody titers using a high-throughput assay," Clin Vaccine Immunol. 2009 Aug;16(8):1105-12. Epub 2009 Jun 17;

4: Garber et al., "Expanding the repertoire of Modified Vaccinia Ankara-based vaccine vectors via genetic complementation strategies," PLoS One. 2009;4(5):e5445. Epub 2009 May 6;

5: Kennedy et al., "Gender effects on humoral immune responses to smallpox vaccine," Vaccine. 2009 May 26;27(25-26):3319-23;

6: Haralambieva et al., "Development of a novel efficient fluorescence-based plaque reduction microneutralization assay for measles virus immunity," Clin Vaccine Immunol. 2008 Jul;15(7):1054-9;

7: Kaufman et al., "Differential antigen requirements for protection against systemic and intranasal vaccinia virus challenges in mice," J Virol. 2008 Jul;82(14):6829-37;

8: Lustig et al., "Combinations of polyclonal or monoclonal antibodies to proteins of the outer membranes of the two infectious forms of vaccinia virus protect mice against a lethal respiratory challenge," J Virol. 2005 Nov;79(21):13454-62;

9: Snyder et al., "Protection against lethal vaccinia virus challenge in HLA-A2 transgenic mice by immunization with a single CD8+ T-cell peptide epitope of vaccinia and variola viruses. J Virol. 2004 Jul;78(13):7052-60; and

10: Cosma et al., "Neutralization assay using a modified vaccinia virus Ankara vector expressing the green fluorescent protein is a high-throughput method to monitor the humoral immune response against vaccinia virus," Clin Diagn Lab Immunol. 2004 Mar;11(2):406-10.

For example, Kennedy et al. relates to a study that examined vaccinia-specific neutralizing antibody responses in a cohort of young, healthy individuals after receipt of a single dose of the Dryvax® vaccine as a means to gather previously unavailable data on gender and racial variations in smallpox vaccine immune responses. The authors utilized the assay of the invention. For example, the reference teaches at page 3320:

2.1.1. Neutralizing antibody assay – The vaccinia-specific neutralization assay developed at the Food and Drug Administration (FDA) was adapted for our use [citing to Manischewitz]. Briefly, heat inactivated serum samples were serial diluted and then mixed with a known concentration of a recombinant, β-galactosidase expressing vaccinia virus for 1 h and then added to Hela cells overnight…After an overnight incubation, cells were

> lysed and β-gal activity levels were monitored using a colorimetric substrate and used as a surrogate marker for virus activity.

In another example, Haralambieva et al. relates to the development of a measles virus-specific neutralization assay using recombinant measles virus engineered to express a fluorescent protein. This reference specifically teaches that "[t]he reproducibility of our assay was found to be lower than or comparable to that shown for the classical PRNT assay and for other neutralization assays, based on reporter gene expression [citing specifically to Manischewitz]." Thus, Haralambieva suggests that its measles-specific assay is not as reproducible as the assay of the invention. This reference is pointed out to emphasize that the present invention, as highlighted in the specification, advantageously demonstrates high reproducibility and that this reproducibility feature has been recognized by others in the art. See e.g., paragraph 121 of the specification.

In yet a another example, Kaufman et al. studied the protective efficacy of administering specific vaccinia antigens against lethal vaccinia virus challenge in mice. It used a virus neutralization assay to measure or assay for neutralizing antibodies. The assay is based on the present invention. Kaufman et al. teaches:

> **Virus neutralization assay.** NAb responses against vaccinia virus were measured using a luciferase-based assay in HeLa cells essentially as previously described for a β-galactosidase neutralization assay [citing to Manischewitz].

It is respectfully submitted that the successful and widespread use of the present invention by those in the pertinent art subsequent to the time of the invention, as clearly evidenced by the above references, should be considered as evidence of the nonobviousness of the present invention. This is further addressed below.

The claims are not rendered obvious by the combination of references:

The present invention is directed to the development of a novel assay to measure protection of cells against virus invasion, i.e., a novel neutralization assay. As taught in the specification, the method as claimed is the only validated alternative method to the classical PRNT assay. As taught in the specification, results obtained using β-gal in the instantly claimed method, are comparable to results obtained with the classic PRNT vaccinia neutralization assays (e.g., paragraph 134). Moreover, as taught in the specification, the claimed method is highly sensitive (e.g., paragraphs 134 and 135), easier to conduct (even with small volumes), faster, and easy to transfer to other

laboratories (e.g., paragraph 121). The assay of the invention was also shown to provide highly reproducible results with low variability (e.g., paragraph 121, 133).

Specifically, the present invention is directed to a method that constitutes an *in vitro* neutralization assay based on the use of a recombinant vaccinia virus vector that is engineered to express an enzyme-based reporter gene, such as β-galactosidase or luciferase. Specifically, claim 1 is directed to a method comprising incubating a mixture comprising at least one cell, a vaccinia virus comprising a reporter gene encoding an enzyme, and a candidate antibody under conditions wherein the vaccinia virus can invade the cell. The method then proceeds by detecting the activity of the enzyme within the cell. The claim further recites that a decrease in the enzyme activity in the cell due to the candidate antibody, relative to a control cell having not been contacted with the candidate antibody, indicates that the candidate antibody decreases invasion of the cell by the vaccinia virus. In addition, the assay is rapid as it can be conducted within 24 hours. The encoded reporter enzyme can be β-galactosidase, luciferase, peroxidase, alkaline phosphatase, or xanthine oxidase, and preferable can be β-galactosidase or luciferase. As reflected in claim 131, the inventive method is predictive of viral lethality *in vivo*. Further, as reflected in claim 139, the inventive method is predictive of the *in vivo* protective efficiency of the antibody against infection with a vaccinia virus.

Hooper does not teach or suggest all of the particularly recited features of the invention. The Examiner contends that Hooper "teaches the production and identification of vaccinia monoclonal antibodies for the purpose of therapeutic treatment (passive immunization) of vaccinia in humans (abstract). Hooper discloses that potential targets for poxvirus therapeutics, monoclonal antibodies, were generated in mice and tested for their ability to neutralize virus and protect mice from challenge." Page 5 of the Office Action. However, Hooper discloses only the use of the classical PRNT assay in its efforts to identify and evaluate the vaccinia monoclonal antibodies. And, as is clear from the specification and the references indicated above, the present invention represents a significant improvement over the PRNT assay. In particular, a key advantage of the present invention is that it is a rapid assay whereby the assay is completed within 24 hours. This departs significantly from the PRNT assay of the prior art, and in turn, Hooper, which utilizes the PRNT assay of the prior art. Indeed, the specification teaches at paragraph 28 that:

> One of the key assays to assess humoral immune responses in vaccinees and the potency of VIG preparations is a vaccinia neutralization assay [PRNT]. The current neutralization assays are all based on plaque reduction neutralizing titers (PRNT). They are slow (4-7 days), require relatively large volume of test articles (since they are conducted in 6-well or 12-well plates), they are also somewhat subjective and operator-dependent since the read-out is visual.

Because Hooper is merely inline with the state of the art over which this invention vastly improves, Hooper should not be considered as a teaching or fair suggestion of the present invention.

In addition, Hooper further distinguishes itself from the present invention because it expressly teaches that "[t]o our surprise, the ability of the MAbs to inhibit plaque formation by vaccinia virus, a standard assay of virus neutralization, ***did not always predict their protective efficacy***. Moreover, the monoclonal antibodies differed in their ability to provide protection depending on the challenge model." By contrast, an unexpected advantage of the present invention is that the claimed assay of the invention is predictive of viral lethality *in vivo* (claim 131) or is predictive of the *in vivo* protective efficiency of the antibody against infection with a vaccinia virus (claim 139). Support for these aspects is provided above. In addition, Applicants submit the draft manuscript entitled "Passive immunotherapies protect WRvFire and IHD-J-Luc vaccinia virus infected mice from lethality by reducing viral loads in upper respiratory track and internal organs," Zaitseva et al. (hereinafter "Zaitseva"), which utilizes whole body bioimaging of recombinant vaccinia virus infections in live mice to predict protection from lethality. On page 18, Zaitseva teaches that the assay of the invention was used to predict *in vivo* protective doses of VIGIV and a cocktail of human monoclonal antibodies ("Sym002"). Zaitseva teaches:

> *In vitro* neutralization assays based on the β-Galactosidase reporter gene expressing vaccinia, demonstrated that the Symphogen cocktail of anti-vaccinia human monoclonal antibodies was 250-500 more effective than VIGIV in blocking infection with WR vaccinia. Therefore the dose ranges selected for the *in vivo* protection studies were between 3 to 30 mg for VIGIV and between 3 to 100 μg for Sym002…(b) prophylactic treatments with either VIGIV (30 mg per animal) or Sym002 (100 μg per animal) protected animals from lethality following challenge with either WRvFire or IHD-J-Luc viruses.

Thus, Zaitseva demonstrates that the assay of the invention, i.e., the "*In vitro* neutralization assays based on the β-Galactosidase reporter gene expressing vaccinia," were used to predict *in vivo*

protective doses of two different neutralization agents which protected animals from lethality following virus challenge.

Hooper does not teach or fairly suggest these features of the present invention because it does not use the novel assay of the invention, but rather the prior art PRNT assay, which according to Hooper's own teachings, was predictive of protective efficiency.

In attempting to cure the deficiencies of Hooper, the Office turns to Auewarakul. The Office concludes at page 5 that:

> It would have been obvious to have modified Hooper's method by using methods known in the art to improve the speed of the assay and also decrease the cost of performing plaque reduction neutralization testing. One would have been motivated to use a reporter virus in a neutralizing antibody assay using flow cytometry as a measurement tool, such as the assay described by Auewarakul in order to test the many antibodies of Hooper with greater speed and reduced costs (see abstract).

Applicants respectfully disagree. Auewarakul—whether taken alone or combined with Hooper—does not cure the deficiencies of Hooper. That is, Auewarakul—whether or not combined with Hooper—does not teach or fairly suggest or even contemplate all of the elements of the presently claimed invention. The assay of the present invention, as instantly recited in the claims, is a rapid neutralization assay that can be completed within 24 hours. By contrast, Auewarakul requires several days or longer to be completed and is thus, not a rapid assay under the present claims. Indeed, Auewarakul requires up to 7 days to be completed (e.g., see Figure 1).

Further, Auewarakul relates to a recombinant HIV-GFP reporter construct, not a vaccinia construct, and in particular, not a vaccinia construct which expresses an enzyme reporter gene, such as β-galactosidase or luciferase. Thus, Auewarakul does not teach or suggest the present invention.

Moreover, the skilled artisan would not have been motivated to modify Hooper with Auewarakul in the first place because Auewarakul would not have accomplished the two stated objectives "to improve the speed of the assay and also decrease the cost of performing plaque reduction neutralization testing." First, as mentioned previously, Auewarakul's method is significantly slower than the assay of the invention, requiring up to 7 days to be completed (e.g., see Figure 1). In more detail, Auewarakul requires the tested sera to be mixed with the recombinant virus and incubated overnight, followed by a media change and incubated for another 3 days. After

this time, the cells were then analyzed by flow cytometry for neutralization activity (page 141, left to middle columns). Thus, the Auewarakul's assay is conducted over a period of 4 days. Auewarakul also conducts a 7-day assay prior to analyzing the nuetralization by flow cytometry (page 141, middle to right columns). The specification teaches that the classical PRNT assays require 4-7 days (e.g., paragraph 28). Thus, Auewarakul's assay would clearly not have accomplished the objective stated in the Office Action of improving the speed of the assay. Moreover, flow cytometry equipment is expensive and adds operating costs to laboratories. Thus, Auewarakul's assay also would not appear to have accomplished the stated objective of reducing the costs of a PRNT assay. Accordingly, Applicants respectfully submit that the skilled person would have completely lacked the necessary motivation to modify Hooper with Auewarakul's assay.

The Office then turns to Dominguez for further support of its obviousness rejection. It states:

> Although Auewarakul's reporter virus is an HIV construct, it would have been obvious to have used Dominguez' green fluorescent protein (GFP) recombinant vaccinia virus that permits early detection of infected cells by flow cytometry (abstract). Dominguez uses the construct as an infection tag (page 116, first column, third full paragraph). One would have expected Dominguez' reporter virus to infect Hooper's cells (if not neutralized by antibodies) and express GFP, which would then be detected by flow cytometry.

Apparently, the Office's position is that while it recognizes that Auewarakul relates to an HIV-GFP construct, rather than a recombinant vaccinia construct, one of ordinary skill in the art would have looked to substitute Auewarakul's HIV-GFP construct with Dominguez' vaccinia-GFP construct, which would have allegedly allowed detection of neutralizing antibodies against Hooper's cells via flow cytometry. Applicants disagree.

First, neither Auewarakul nor Dominguez teach the use of enzyme-based reporter constructs. Instead, both Auewarakul and Dominguez rely on the detection of fluorescent protein-based constructs. Auewarakul teaches an HIV-GFP expression construct and Dominguez teaches a vaccinia-GFP expression construct. Thus, neither of the references teach or suggest a recombinant vaccinia expressing a enzymatic reporter for use as a reporter for a neutralization assay.

While the Office Action notes that "Dominguez discloses that a number of marker genes have been inserted in the vaccinia virus genome, and that their utility has been demonstrated in different experimental situations," Applicants submit that each of the references cited by Dominguez was in the context of using enzyme genes in the construction of cloning vectors, rather than in the design of vectors for the specific purpose of their use in neutralization assays. Thus, Dominguez does not teach or suggest the specific vectors used in the inventive method.

Instead, Dominguez teaches the construction of a recombinant vaccinia expressing GFP for detection of cells by flow cytometry. The construct as taught by Dominguez is merely used "as an infection tag" and "is useful for studying tropism." There can be no suggestion to use the Dominguez' method as an vaccinia neutralization assay, or that the method could be used to provide a vaccinia neutralization assay that is predictive of results from an *in vivo* challenge assay or which could be validated against a PRNT assay. Dominguez merely teaches "the usefulness of GFP as an infection marker" (p. 120), not as a marker that would be useful in neutralization assays that are predictive of *in vivo* virus lethality or of protective efficiency as claimed. In determining tropism, there can be no motivation to include a "candidate agent" in the assay that would modulate binding or invasion of the virus into the cell because by doing so would negate the purpose of detecting tropism. That is, the candidate agents of the invention neutralize vaccinia virus, i.e., do not enter the target cells. Dominguez monitors tropism, of which the objective is to monitor entry of the virus into specific cells. The candidate agents of the invention would interfere with this process, and thus, would tend to negate the entire purpose of Dominguez.

Neither Briskin nor BD Biosciences cure the deficiencies of Hooper, Auewarakul and Dominguez. Brikin and BD Biosciences were merely cited as allegedly teaching the specific limitations of dependent claims 132, 133, 134 and 135. The Office admits that Briskin's "teachings are an example of the technology available at the time of the invention with regard to high throughput screening" but are "directed to different products." Thus, Briskin, by admission of the Office, is merely cited as it generally relates to high throughput screening methods and thus, does not teach or suggest the specific assay of the invention. Likewise, BD Biosciences merely is a generalized guide for using flow cytometry and has nothing to do with the specific assay of the

invention. Accordingly, Briskin and/or BD Biosciences do not cure the deficiences of Hooper, Auewarakul and Dominguez.

Even if, *arguendo*, the combination of references taught or suggested all of the elements of the claims—which is not being admitted here—a person having ordinary skill in the art would not have been motivated to combine the references. This is in part for the reasons already given above—i.e., because modifying Hooper with Auewarakul and/or Dominguez would be contrary to the objectives and/or purpose stated in Auewarakul and Dominguez.

Moreover, one of ordinary skill in the art, armed with Hooper and the state of the art at the time of the invention—despite the noted disadvantages of the PRNT assay—would not have likely sought alternative ways to conduct a neutralization assay because the state of the art at the time of the invention considered the PRNT method to be the "gold standard." Applicant provided with the last Response of June 24, 2010 a copy of the "Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses" published by the World Health Organization in 2007 (hereinafter referred to as "the Guidelines"). The Preface and Background section (page vii) of the Guidelines discusses the use of the PRNT assay:

> In an attempt to make inter-laboratory information more directly comparable, WHO and PDVI initiated a program to harmonize the procedures used for the plaque-reduction neutralization test (PRNT). The PRNT is the most common assay used to measure neutralizing antibody. The presence of antibody is believed to be most relevant for determining protective anti-DEN virus (DENV) immunity. While other neutralizing antibody assays are being considered for use in large scale vaccine field trials, ***the PRNT is still considered to be the laboratory standard against which other neutralizing antibody assays should be compared*** (Martin et al., 2006; Vorndam and Beltran, 2002). [emphasis added]

On page 1 of the Guidelines, it is noted that

> The virus PRNT remains the most widely accepted approach to measuring virus-neutralizing and protective antibodies. Newer assays measuring virus neutralizing antibodies are being developed and will be briefly discussed later in this document.

Page 5 of the Guidelines discuss other tests, and their shortcomings:

> A variety of serological tests have been used to measure anti-flaviviral antibody. These tests include the hemagglutination-inhibition test, complement fixation test, fluorescent antibody test, enzyme-linked immunosorbent assay (ELISA), and PRNT. Each of these tests measures different antibody activities. Only the PRNT measures the biological parameter of *in vitro* virus neutralization and is the most serologically virus-specific test among flaviviruses, and serotype-specific test among dengue viruses, correlating well to serum levels of protection from virus infection. ***Newer tests measuring virus neutralization are being developed, but PRNT remains the laboratory standard against which these tests will need to be validated.*** (page 5, emphasis added)

Therefore, despite the difficulties and problems of the PRNT test, no test discussed in the Guidelines had been developed by 2007 to supplant the PRNT assay or validated against the PRNT assay—until the assay of the present invention. And, the success and significance of the assay of the invention is evidenced by its now widespread use and acceptance of the scientific community working in the field of study pertaining to the present invention (see above references). Therefore, the skilled person would not have reasonably expected to modify Hooper with the assay of Auewarakul using Dominguez's vector and be successful in developing the rapid assay of the invention, which is predictive of viral lethality *in vivo* and is predictive of the *in vivo* protective efficiency of a candidate agent against a vaccinia virus infection.

The present invention is directed to the development of a novel assay to measure protection of cells against virus invasion; a novel neutralization assay. ***As taught in the specification, the method as claimed is the only validated alternative method to the classical PRNT Assay.*** As taught in the specification (e.g. page 46, beginning at line 26) ***results obtained using beta-gal in the instantly claimed method, are comparable to results obtained with the classic PRNT vaccinia neutralization assays.*** Moreover, as taught in the specification, the high throughput technology makes the claimed method highly sensitive, easier to conduct (even with small volumes), faster, and easy to transfer to other laboratories.

The usefulness of the instantly claimed assay and its adaptability to a high-throughput method has been further demonstrated in Kennedy et al., which demonstrates the ease with which the method of the invention can be converted to a high throughput screening method. In addition, the usefulness of the inventive assay in predicting *in vivo* doses of neutralizing agents that are

effective in protecting against vaccinia lethality are demonstrated in Zaitseva. In another publication Zaitseva et al., Application of bioluminescence imaging to the prediction of lethality in vaccinia virus-infected mice. *J. Virol.*, 83:10437-47 (hereinafter "Zaitseva II"). It is clear from the Guidelines discussed above that the development of an assay to determine the protection afforded by antibodies or other agents could only be achieved with the PRNT assay and that any other "newer test measuring virus neutralization" first "will need to be validated." As evidenced throughout the specification, as noted above, and in the post-filing references mentioned herein, including Kennedy et al., Kaufman et al., Haralambieva et al., Zaitseva, and Zaitseva II, the assay of the present invention has been validated against PRNT and shown to have many advantages, as indicated above.

Further, Applicants respectfully request that the post-invention publications listed above be weighed as objective evidence of nonobviousness. The MPEP instructs at Section 2145:

> Rebuttal evidence may include evidence of "secondary considerations," such as "**commercial success, long felt but unsolved needs**, [and] failure of others." *Graham v. John Deere Co.*, 383 U.S. at 17, 148 USPQ at 467. See also, e.g., *In re Piasecki*, 745 F.2d 1468, 1473, 223 USPQ 785, 788 (Fed. Cir. 1984) (commercial success). Rebuttal evidence may also include evidence that the claimed invention yields unexpectedly improved properties or properties not present in the prior art. Rebuttal evidence may consist of a showing that the claimed compound possesses unexpected properties. *Dillon*, 919 F.2d at 692-93, 16 USPQ2d at 1901. A showing of unexpected results must be based on evidence, not argument or speculation. *In re Mayne*, 104 F.3d 1339, 1343-44, 41 USPQ2d 1451, 1455-56 (Fed. Cir. 1997) (conclusory statements that claimed compound possesses unusually low immune response or unexpected biological activity that is unsupported by comparative data held insufficient to overcome *prima facie* case of obviousness). Rebuttal evidence may include **evidence that the claimed invention was copied by others**. See, e.g., *In re GPAC*, 57 F.3d 1573, 1580, 35 USPQ2d 1116, 1121 (Fed. Cir. 1995); *Hybritech Inc. v. Monoclonal Antibodies*, 802 F.2d 1367, 1380, 231 USPQ 81, 90 (Fed. Cir. 1986). It may also include **evidence of the state of the art**, the level of skill in the art, and the beliefs of those skilled in the art. See, e.g., *In re Oelrich,* 579 F.2d 86, 91-92, 198 USPQ 210, 214 (CCPA 1978).

It is respectfully submitted that the post-invention publications provided herewith demonstrates that the state of the art includes publications that show the immediate acceptance and utilization of the assay of the invention by those of ordinary skill in the art pertaining to the present

invention. Although these post-invention references do not *per se* constitute commercial success (i.e., not evidence of sales or profits), they are analogous to commercial success in that those of ordinary skill other than the inventors began using the assay of the invention. This is even more significant when considering that, even as of 2007, as indicated by the Guidelines, the PRNT assay was clearly regarded as the "gold standard" neutralization assay. Moreover, the fact that the PRNT assay—while being the "gold standard"—had known disadvantages (e.g., slow), and the fact that the present inventive neutralization assay did not appear before the time of the invention, strongly suggests that there was a "long felt but unsolved need" in the art that simply was not solved until the present inventors developed and tested and validated the instantly claimed assay.

Applicants respectfully submit that, in view of at least the above arguments and amendments, the presently claimed invention of claim 1 is not obvious in view of the combination of Hooper, Auewarakul and Dominguez. By extension, each of the remaining claims all of which directly or indirectly depend from claim 1, too are not obvious in view of the cited references. Accordingly, Applicants request reconsideration and withdrawal of the Section 103 rejections.

CONCLUSION

In view of the amendments and remarks herewith, the application is believed to be in condition for allowance. Applicants respectfully request entry of this paper, favorable reconsideration and withdrawal of the objections to and rejections of the application, and prompt issuance of a Notice of Allowance. If a telephone conversation with Applicants' attorney(s) would help to expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned.

Respectfully submitted,

Date: March 8, 2011

/Gabriel J. McCool/
Gabriel J. McCool
Reg. No. 58,423
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
Phone (203) 975-7505
Attorneys/Agents for Applicant(s)